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DISCUSSION ON THE PRECEDING PAPER

DR. G. L. M. MCELLIGOTT (the President) said he was interested to hear that the false positive Kahn reactions might possibly be caused by temperature, because this might explain certain discrepancies noticed between Kahn and Wassermann reactions during the recent cold spell. He was interested in what had been said about atypical pneumonia and had himself observed a case in which an *x*-ray opacity in the lung gradually vanished with the decline to negative of the false positive Wassermann.

Much had been said about false positives; he would also like to hear the speaker's views on false negatives. In many cases presented for an opinion the Wassermann was repeatedly negative, contrary to clinical findings.

DR. I. N. ORPWOOD PRICE said that when he thought of all the diseases which were said to be the cause of false positive Wassermann reactions he wondered why they bothered to do such a test. Most of this evidence came from abroad. Not very much had been produced in this country to show that false positive reactions were obtained from the diseases enumerated, and he felt that the many false positives reported must be due to faults in technique, not only in the Wassermann but also in the Kahn test. There was, moreover, little doubt that the Kahn antigen produced in America fifteen years ago was a better product than that produced now. He wondered whether commercial firms manufactured their Kahn antigen by mass production methods, which yielded an antigen more liable to give false positive reactions. In his own experience a true biological false positive was uncommon. He wondered what all this evidence really meant. He worked with clinicians who were keen, and who told him promptly if a mistake had been made. He was grateful for this co-operation; but if these false positives were so common he would have been told about them and would have had to do something. He had not studied a series of particular diseases; in fact he could think of only three types of cases which might have any bearing on the matter. First, mononucleosis; and in this disease he knew of only one patient who gave a false positive reaction. This remained positive for about a fortnight, during which five different specimens of blood were taken; each test gave a very weak positive result except the last, which was negative. Secondly, he estimated that he had examined 10,000 routine specimens from pregnant patients. He had expected 3 per cent. to be positive. In fact, up to the present, the rate had been about 0·05 per cent. Thirdly, he had thought that high fever might give a false positive reaction, and when Mr. King was at Westbury he supplied Dr. Price with specimens of blood from patients who were undergoing high fever treatment. The specimens were taken at the height of the fever, and of 80 tests all were negative except one, an American negro suffering from syphilis. He could not help thinking that a lot of nonsense was talked about biological false positives. He would not say they did not occur, but that if they did the numbers would appear to be very small.

COL. L. W. HARRISON wished to endorse what Dr. Price had said about the importance of technique. He believed that many false positives were the result of bad technique. A number of tests called "Harrison-Wyler," carried out in this country, had given very different results with the same sera from those obtained in the Venereal Diseases Reference Laboratory, where the H-W method was the standard. Some people who said they practised that method (and he was sure this applied to the Kahn and other well-known methods) did not practise it in all its details. He would like to have sent many of the serologists whose test methods had been compared with the standard used in the Reference Laboratory to school with Dr. Wyler, who "could distinguish and divide a hair twixt north and south-west side." Dr. Wyler was unhappy if anyone walked into his laboratory smoking a cigarette, because he feared the anti-complementary effect of a flake of tobacco ash falling into one of his tubes; many serologists might copy with profit his care of glassware and of his complement sera between titration and use in the test-proper.

He thought that statements made by many authorities that pregnancy did not tend to cause false positives was based on slender evidence. Good evidence that it did so could be found in the Report of the first Serum Conference at Copenhagen, in which there are reports of tests carried out in separate laboratories between the date of the Paris Conference and that held in Copenhagen. Reports of the State Serum Institute and of the Warsaw Institute recorded many positives in pregnancy under the heading of patients without clinical evidence or history of syphilis. His own experience in the many comparisons of serum test methods carried out by the Ministry of Health's Venereal Disease Reference Laboratory between the two wars was that a shaky

test would be shown up by pregnancy sera more readily than by the normal sera commonly used for controls.

If two or more tests were used, one should be a complement fixation test. At the Serum Conference in Copenhagen in 1928 he had argued this point with Dr. Kahn, who had contended that one of the tests need not be a complement fixation. The opinion of the delegates was taken and representatives of seventeen of the twenty-one laboratories taking part in the Conference stated their preference for inclusion of a complement fixation test; he believed this view was held more generally today than it was in the early days of the more successful flocculation tests.

There was value in doing the test quantitatively in cases under treatment and when there was any suspicion that the result was false. He would agree also that in any case of doubt a serum should be submitted to one or more other laboratories.

DR. T. E. OSMOND agreed with Dr. Orpwood Price and Colonel Harrison on the question of technique. He thought that the Wassermann was the most abused test in pathology, and in this country it was performed incorrectly more often than any other test. Dr. Thomson had referred to the fact that a positive Wassermann did not necessarily mean syphilis; yet hundreds of doctors wrongly thought otherwise.

He was interested in what Dr. Thomson had said about the effect of protein. If a high protein intake was likely to produce a false positive, this should become less and less frequent in present circumstances! With regard to the number of false positives, he thought that possibly Dr. Price had misunderstood what Dr. Thomson had said. The fact that there were scores of diseases that might give, or were said to give, false positives did not mean that false positives were very common. Many of these diseases were tropical and did not occur in this country, and many were uncommon; any clinician should hesitate to accept a positive reaction in the presence of a disease other than syphilis. What was happening was that many pathologists had found a positive reaction in such and such a disease and reported it in the literature, implying that such and such a disease gave a false positive Wassermann reaction. The next writer quoted him as saying *X* disease produced a false Wassermann reaction and that was how the reputation had grown. A few years ago he had investigated carefully a considerable number of specimens from cases of scarlet fever without getting even one positive Wassermann reaction among them. One must distinguish between the false positive due to some definite disease other than syphilis, and the false positive which occurred in an apparently healthy person; the two things were quite different. If the patient was suffering from a particular disease one could wait until he had recovered and keep on testing his blood. One would expect to find some differences in a patient who had normal health; in syphilis the Wassermann might be double plus almost indefinitely, and that was where the quantitative test was valuable. False reactions did not usually give a very high titre, and on repetition there might be a change up or down. If it was up it was important, but if it was down the verdict was against syphilis.

He had recently written a critical review of the literature on this subject; he would not repeat all he had said, but there were one or two points worth mentioning now. One was that there was an antigen known as "cardiolipin" being developed in the United States at the present time, and its use might eliminate some of the false positive reactions. It was a good idea to do two tests, and do them on separate specimens of serum taken from the same blood. The blood came in a tube from the clinic to the laboratory, and he insisted on his technician putting up two samples of each specimen, one for the Kahn and one for the Wassermann, so that any question of a mixture of specimens in the laboratory would be discovered.

Until the Ministry of Health issued its own supply of rabbit serum, many pathologists were using horse serum amboceptor; they did not realize that this led to false positive reactions.

Many people now believed the Kahn verification test was useless. When he started doing the Kahn test in 1922 he obtained his Kahn antigen directly from Dr. Kahn and it was very good. Subsequently he made his own antigen from dried heart powder, and that apparently worked quite well. During the war when he was responsible for a great many blood tests he was very uneasy about the Kahn antigen supplied to the Army, and was quite sure that some of it was of poor quality. Recently he had again obtained a considerable supply of Kahn's own antigen and had found it excellent. It was insensitive rather than oversensitive. He was not prepared to give an opinion on the antigen being marketed in this country nowadays. In ninety-nine cases out of a hundred a positive reaction without clinical signs was not an emergency, and a decision could wait for three or even six months. It was better to wait and do a series of tests rather than come to a wrong decision and start treating a non-syphilitic patient for syphilis. A positive reaction which did not agree with the facts should be viewed with suspicion.

COL. FAWKNER-CORBETT was critical of the suitability of some pathological specimens and their submission. It would be a great advantage if all new house surgeons were taught the correct way to take specimens. The next point was to get the right name or identification number on the specimen.

He was in complete agreement with what had been said with regard to the Kahn antigen. He had had cases in which spirochætes had been found, the Wassermann had been positive, and the Kahn had been completely negative; consequently the Kahn test with him had fallen into disrepute. He also agreed on the question of having a flocculation test controlled by a fixation test. A quantitative estimation was of value in the latter test. He had known patients giving a positive result in whom there were no clinical signs. In one he himself had corroborated a previous positive finding; in the other his own positive

result had been confirmed at another laboratory. There were no clinical signs in either patient, but in each there was a history of syphilis in a maternal grandmother. He suggested that the Biblical reference to the sins of the fathers being transmitted to the third and fourth generation be kept in mind. Were the positive results in these two cases really false? Might not something have been transmitted which could cause a positive Wassermann reaction although there was no clinical evidence?

Two other cases had come before him which, lest clinicians rely too much on their house surgeons, might be borne in mind. The Wassermann reaction had been found strongly positive in sera from two patients, but, according to the medical officers, in neither were there any clinical signs. The specimens were re-tested and the same results were obtained. He therefore went to see the patients himself, and found Hutchinson's teeth and scars of old interstitial keratitis in both cases. A thorough clinical examination was essential in trying to make certain whether a positive Wassermann reaction was true or false.

DR. H. M. HANSCHELL said that most of the reports of a positive Wassermann test in non-syphilis cases had come from the tropics, and the reliability of many of these tests had been more or less rightly disputed. Yaws hardly needed mention —it was most probably syphilis. There was the important and common tropical disease, malaria, and this infection had already, in these post-war days, passed unrecognized as such in men and women returned from war service in the tropics. It was a fact that malaria very often gave a clear positive Wassermann during the fever and sometimes for five or six weeks after its cessation. In consequence, some of these cases had been suspected of syphilis when blood tests had been done in the non-febrile period, at which time malaria parasites were absent from the peripheral blood.

DR. NEVILLE MASCALL said he felt he had been fortunate in the pathologists with whom he had associated. He started off with Dr. Osmond and passed on to Dr. Orpwood Price, and now he was with Dr. Thomson. He had come to realize the value of close contact with the pathologist. For a time he was in the position where he was absolutely unable to get into touch with his pathologist and had to take his reports by post. Most of them came back anticomplimentary. It was claimed that this was due to the results of posting. There must be many people in this country who were working under the same difficulties. As a clinician he would say that he thought the time had come when there should be a standardization of the reports of tests. Each pathologist's report was more or less different; although there was a standard available for reports, it was not used.

Recently he had seen two examples which he thought were false positive reactions. The patients had now been watched for a period of six months and had both become negative. One was a man who had recently been vaccinated. His Wassermann was never stronger than 1 plus; his Kahn was positive. There were no clinical signs of syphilis. He was closely watched and the Wassermann came down slowly, and the Kahn was also now negative. The other was a patient with lymphatic leukæmia for whom a splenectomy had been done in the Army, and he gave a positive Wassermann and Kahn reaction. He showed no evidence of syphilis, and after about three months his Wassermann and Kahn became negative. These two cases could be classified as giving false positive results, because the Wassermann and Kahn test were repeated so often that an error in technique would have been discovered.

The serological reactions of pregnant women had been a source of worry to him and he was afraid he did not agree with Col. Harrison. He thought, on the whole, that the serum of pregnant women tended towards negativity. He had seen quite a number of women with negative Wassermann reactions who were delivered of syphilitic children. Something must have suppressed the disease; otherwise they would have given a positive result. There were many patients who had had the minimum amount of treatment to make their blood test negative, but probably not sufficient to keep it negative. If there were a tendency to positivity during pregnancy, one would expect this type of case to revert back to being positive, but he never saw this happening. If there was the least suspicion of doubt regarding pregnant women, it was safer to treat them. He was opposed to treatment without definite evidence but if the husband was a known syphilitic and his wife had negative serological tests then that wife should be treated during her carrying period. Full treatment was surely worth while if the birth of a congenital syphilitic child could be prevented. He strongly advocated the treatment of all known syphilitic mothers during each pregnancy, whether their blood was negative or positive.

DR. R. R. WILLCOX said that, if yaws was, as some suggested, the same disease as syphilis, the so-called "false positives" from this disease were in fact true positives. Within recent weeks he had seen a number of West Africans, and of the last fifteen no less than five had strongly positive Wassermann and Kahn reactions with no clinical or historical evidence of syphilis. Yaws being a disease of childhood, it was not always possible to get a history, but one of these sero-positive patients gave a history of yaws at the age of five. Dr. Thomson had quoted Butler on the subject of the relationship of yaws and syphilis, but it should be remembered that this author had been to some trouble to prove that yaws and syphilis were the same disease. This was partly wishful thinking as, if this could be so proved, then a case could be made to show that syphilis was not brought back from America by Columbus' crew but, on the contrary, had been imported into America by the slaves.

COL. L. W. HARRISON said that on the question of pregnancy he thought he could agree with Dr. Mascall. He emphasized that a shaky technique

would bring out false positives in pregnancy. Pregnancy might tend to weaken the real syphilitic reaction; pregnancy might in itself be a mild treatment for syphilis.

SQUADRON-LEADER NOBBS said that he had recently seen two warrant officers in the Royal Air Force who had positive Wassermann reactions and a negative Kahn test. The pathologist had suggested that these results might be due to the blood cholesterol content. This was tested and found to be high, and he wondered if there was anything in the suggestion.

DR. R. B. TAMPI said that in India, where most of the diseases listed by Dr. Thomson were endemic, the false positive Wassermann reaction was a problem, particularly in areas where there was a great incidence of malaria. It was difficult for him to say whether these positives were technically or biologically false positives, but it had been his experience that one did get frequent positive reports in malaria in the acute stages and for about four weeks following the attacks. Positive results in chronic malaria were also seen.

Of all the diseases which gave rise to false positive reactions in that part of the world, he would consider yaws most important Next to yaws came leprosy, particularly in what was called the lepromatous type; it was not so common in the neural type. It was unfortunate, because in certain types of leprosy the skin lesions were difficult to differentiate from the skin lesions of syphilis. The third disease was lymphogranuloma inguinale. He had found ten persons with this condition who gave a false positive which after a time became negative. He had not had much experience in the other conditions mentioned.

DR. THOMSON, in reply, said he was glad to have the opinion of pathologists on the frequency of false positive reactions, because he had felt rather doubtful in his own mind about their frequency and the high incidence reported in the U.S.A. literature. He was averaging about 4,000 Wassermann tests a year, and it was very seldom that he obtained a false positive reaction. Occasionally he obtained a doubtful (±) reaction which he did not place in the category of a false positive reaction because when the tests were repeated they became negative. He was relieved that his brother pathologists supported his opinion that false results were not so frequent as was stated in the literature. Nevertheless, he felt that the possibility of false biological positives must be recognized and the clinicians must be on the watch for them.

He agreed with Dr. Orpwood Price that the powdered beef heart material as supplied commercially for the Kahn test was very unsatisfactory. He had tried making up batches himself and had found that only one out of three or four batches had been satisfactory, which was disappointing to the technicians preparing the Kahn antigen. For this reason he was glad that there was a central laboratory where one could get the standard reagents. He thought that in the days when pathologists made their own antigens they got more false positives than they did now when using specially prepared standard reagents. He would recommend laboratories as far as possible not to make their own, but to get it from a central laboratory.

It was very difficult to explain the occurrence of negative Wassermann in cases of syphilis, where syphilis was definitely diagnosed. Sachs, who was a master of the serology of the Wassermann test, said that, although flocculation tests were usually more sensitive than the Wassermann reaction, occasionally the opposite might obtain. He said that the complement fixation and flocculation tests results were the consequences of the same alteration of the globulins, which might be caused either indirectly or specifically, although flocculation tests were usually more sensitive. The complement fixation occurred best when the antigen-antibody complexes were in the state of development, and this depended to some extent on optimum proportions similar to those intended to produce a flocculation reaction. On the other hand, complement fixation might be inhibited by non-specificity of the components. Because of these peculiarities, both Wassermann and Kahn tests should be used in the diagnosis of syphilis.

He could not answer the question whether a high blood cholesterol might cause false positive reactions. It was an interesting point, and there was probably something in it; it should be further investigated and the results published.